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# Calcium-activated membrane depolarization via modulation of chloride efflux from parietal cells during gastrin stimulation

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In the isolated parietal cell the following observations were made: gastrin led to an increase in cytoplasmic free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) even in the absence of medium  $\text{Ca}^{2+}$  and to transient membrane depolarization in both the absence of the medium  $\text{Ca}^{2+}$  and the fura-2-loaded cell. The incorporated  $\text{Ca}^{2+}$  chelator BAPTA inhibited the gastrin-induced membrane depolarization. The magnitude of depolarization caused by gastrin was unchanged on removal of medium  $\text{Na}^+$ . Furosemide but not tetraethylammonium inhibited the gastrin-induced depolarization. The results suggest that the  $\text{Ca}^{2+}$  released from the store(s) induces membrane depolarization, possibly via modulation of a  $\text{Cl}^-$  efflux across the luminal surface during gastrin stimulation.

Gastrin;  $\text{Ca}^{2+}$  release; Depolarization;  $\text{Cl}^-$  efflux; (Parietal cell, Guinea pig)

## 1. INTRODUCTION

In mammalian gastric parietal cells the intracellular  $\text{Ca}^{2+}$ -mobilizing hormone is thought to be gastrin, since it causes an increase in aminopyrine accumulation or oxygen consumption (as an index of acid secretion) and in  $[\text{Ca}^{2+}]_i$  in both the absence of medium  $\text{Ca}^{2+}$  and the presence

of a  $\text{Ca}^{2+}$ -channel blocker [1–3], thus differing from histamine, which produces cyclic AMP, and from cholinergic muscarinic stimulation, which increases  $\text{Ca}^{2+}$  entry from the extracellular space [3,4]. An ATP-dependent and inositol trisphosphate-sensitive  $\text{Ca}^{2+}$  pool is located in the apical portion of the guinea pig parietal cell that comprises tubulovesicular elements and secretory canaliculi and is connected with microfilaments [5]. However, the gastrin-elicited  $\text{Ca}^{2+}$  metabolism has not yet been substantiated including the question of how  $\text{Ca}^{2+}$  released from the store(s), as an intracellular signal transducer, leads to acid secretion from the gastric parietal cell. One possibility is that  $\text{Ca}^{2+}$  regulates  $\text{K}^+$  permeability and the subsequent activation of  $\text{H}^+$ ,  $\text{K}^+$ -ATPase, implying a direct control of  $\text{Ca}^{2+}$  on  $\text{K}^+$  channels [6]. On the other hand, the stimulation activates a  $\text{Cl}^-$  component in the secretory membrane and not in the basolateral membrane of the parietal cell via a  $\text{K}^+$ - $\text{Cl}^-$  pathway. The  $\text{Cl}^-$  component in the

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**Abbreviations:** fura-2, 1-(2-(5'-carboxyoxazol-2'-yl)-6-aminobenzofuran-5-oxy)-2-(2'-amino-5'-methylphenoxy-ethane-*N,N,N',N'*-tetraacetic acid; BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid; diS-C<sub>3</sub>-(5), 3,3'-dipropylthiodicarbocyanine; Mops, 3-(*N*-morpholino)propanesulfonic acid; quin-2, 2-(2-amino-5-methylphenoxy)methyl-6-methoxy-8-aminoquinoline-*N,N,N',N'*-tetraacetic acid

stimulated state results from the addition of  $\text{Cl}^-$  conductance in the  $\text{H}^+$ - $\text{K}^+$  pump-associated membrane [7,8]. It is well known that  $\text{Ca}^{2+}$  regulates  $\text{K}^+$  and  $\text{Cl}^-$  currents via the opening of specific channels in many exocrine cells [9,10]. Therefore, the aim of this study has been focused on defining the relationship between a functional role for  $\text{Ca}^{2+}$  released from the store(s) and the  $\text{K}^+$  or  $\text{Cl}^-$  pathway during gastrin stimulation.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The sources of some of the reagents mentioned below have been given previously [1,2,5,11]. diS-C<sub>3</sub>-(5), from a stock solution of 1 mM in dimethyl sulfoxide, was obtained from Molecular Probes (USA). Aequorin was purchased from Dr Blinks, whose preparation was lyophilized from a solution containing 1 mg/ml aequorin, 5 mM Hepes and 150 mM KCl (Mayo Foundation, USA). 1 mM fura-2, 10 mM quin-2 and 50 mM BAPTA acetoxymethyl esters in dimethyl sulfoxide were obtained from Dojindo (Japan).

### 2.2. Isolation of parietal cells

Heterogenous gastric mucosal cells from guinea pig gastric mucosa (Hartley, male, 250 g) were prepared as in [2,11].

Enriched cell populations were prepared by Percoll density gradient ultracentrifugation ( $30000 \times g$  for 15 min at  $4^\circ\text{C}$ ) [11]. A fraction enriched in parietal cells was recovered at a density of between 1.043 and 1.050 g/ml. Chief cell contamination was less than  $13.40 \pm 0.32\%$  ( $n = 6$ ).

### 2.3. Measurement of membrane potential

2  $\mu\text{l}$  of 1 mM diS-C<sub>3</sub>-(5) was added to 1.7 ml Mops-Tyrod buffer (in mM: 150 NaCl, 5 KCl, 1  $\text{MgSO}_4$ , 0.2  $\text{KH}_2\text{PO}_4$ , 0.8  $\text{K}_2\text{HPO}_4$ , 10 Mops-Na, 5 glucose, pH 7.4) with (1 mM  $\text{CaCl}_2$ ) or without (0 mM  $\text{CaCl}_2$ , 1 mM EGTA)  $\text{CaCl}_2$  and then 300  $\mu\text{l}$  cell suspension ( $10^5$  parietal cells in Mops-Tyrod buffer) was added to the cuvette at  $37^\circ\text{C}$  (final concentration of diS-C<sub>3</sub>-(5), 1  $\mu\text{M}$ ). The signal was recorded at wavelengths of 620 nm for excitation and 666 nm for emission, both slit widths being 20 nm, using a Hitachi 650-60 fluorescence spectrometer (Japan). The fluorescence changes are reported as a percentage of

the total full-scale fluorescence. For all measurements (including those described in section 2.4), cells were temperature equilibrated with stirring in a thermostatted cuvette at  $37^\circ\text{C}$  for 3 min.

### 2.4. Measurements of cytosolic free $\text{Ca}^{2+}$ concentration ( $[\text{Ca}^{2+}]_i$ )

2  $\mu\text{M}$  fura-2 acetoxymethyl ester was added to a parietal cell suspension ( $10^6$  cells/ml) in RPMI-1640 medium containing 10 mM Mops and 25 mM  $\text{NaHCO}_3$ . The cell suspension was incubated for 15 min at  $37^\circ\text{C}$  [13]. After loading of the dye fura-2, the cell suspension was rinsed twice and  $0.25 \times 10^6$  cells were resuspended with 0.5 ml of low  $\text{Na}^+$  and high  $\text{K}^+$  Mops-Tyrod buffer (40 mM  $\text{Na}^+$ , 100 mM  $\text{K}^+$ ) in the presence (1.0 mM  $\text{Ca}^{2+}$ ) or absence (prepared by omitting  $\text{CaCl}_2$  and adding 1 mM EGTA) of medium  $\text{Ca}^{2+}$ . Fura-2 fluorescence was detected using a CAF-100 fluorescence spectrometer (Japan Spectroscopic). The emission wavelength was set at 500 nm while the excitation wavelength was obtained from the 340/380 nm ratio [12] or maintained at 380 or 340 nm for the entire measuring period.  $[\text{Ca}^{2+}]_i$  values were calculated from fura-2 ratios by the equation in [12]. The method of quin-2 (20  $\mu\text{M}$ ) loading was very similar to that of fura-2 [1,2]. Quin-2 fluorescence was recorded with a Hitachi 650-10LC fluorescence spectrometer. The excitation and emission wavelengths were 339 and 492 nm with 4 and 10 nm bandwidths, respectively.  $[\text{Ca}^{2+}]_i$  was calculated as in [13]. Aequorin loading was performed as in [14,15]. Parietal cells were incubated ( $5 \times 10^6$  cells/150  $\mu\text{l}$ ) at  $24^\circ\text{C}$  for 6 min in Mops-Tyrod solution with 1 mM ATP and 2 mM EGTA following the addition of 10  $\mu\text{l}$  aequorin (0.3 mg/ml aequorin in 5 mM Hepes + 50 mM KCl + 7 mM EGTA). Dimethyl sulfoxide was added stepwise during the incubation to a final concentration of 6%. Incubation was terminated by the addition of 900  $\mu\text{l}$  Mops-Tyrod solution with 5 mM EGTA and 1 mM ATP followed by centrifugation at  $12000 \times g$  for 15 s. The resultant cell pellet was resuspended with 10 ml Mops-Tyrod solution.  $\text{CaCl}_2$  was added to 1 ml cell suspension ( $5 \times 10^5$  cells/ml) to a final concentration of 1 mM in a cuvette and gastrin added after the signal from the  $\text{Ca}^{2+}$ -aequorin complex outside the cells had disappeared. Aequorin luminescence was determined with a platelet ionized calcium ag-

gregometer (Chrono Log, USA) with constant stirring at 37°C.  $[Ca^{2+}]_i$  was calculated as in [14].

### 3. RESULTS

diS-C<sub>3</sub>-(5) is a lipophilic cation whose fluorescence is quenched when it accumulates inside cells. Thus, when this dye is added to a cell suspension, the distribution between the cell-bound and free dye in the medium depends on the membrane potential. Depolarization causes a decrease in the amount of cell-associated diS-C<sub>3</sub>-(5) and the fluorescence of diS-C<sub>3</sub>-(5) increases when it is released from cells [16]. As shown in fig.1, addition of gastrin ( $10^{-8}$  M) to parietal cells suspended in  $Ca^{2+}$  (1 mM)-containing medium increased the fluorescence signal of diS-C<sub>3</sub>-(5) in a transient manner which appeared to represent depolarization and subsequent re-polarization of the cell. The depolarization transient brought about by gastrin was shown even in the absence of

medium  $Ca^{2+}$  (0 mM  $Ca^{2+}$ , 1 mM EGTA) and was similar in magnitude to that observed in the presence of medium  $Ca^{2+}$ . In contrast, the  $Ca^{2+}$  ionophore ionomycin-induced membrane depolarization was mainly dependent on medium  $Ca^{2+}$ . However, the small but transient depolarization (by ionomycin) was also demonstrated even in the absence of medium  $Ca^{2+}$ . Higher concentrations of ionomycin (2.5–5  $\mu$ M) in the presence of medium  $Ca^{2+}$  failed to re-polarize the cells, possibly owing to no  $Ca^{2+}$  gradient being present. The acetylcholine analogue carbachol-elicited depolarization disappeared in the absence of medium  $Ca^{2+}$  which was similar to the  $[Ca^{2+}]_i$  change that required medium  $Ca^{2+}$  [3].

As shown in fig.2, the increase in  $[Ca^{2+}]_i$  elicited by gastrin was almost wholly due to the intracellular  $Ca^{2+}$  released from the store(s), since the omission of medium  $Ca^{2+}$  failed to decrease the signals measured with fura-2, quin-2 and aequorin. In the case of measurement with fura-2 or quin-2, the average resting level of  $[Ca^{2+}]_i$  of the parietal cell was 170 and 200 nM, respectively. Gastrin

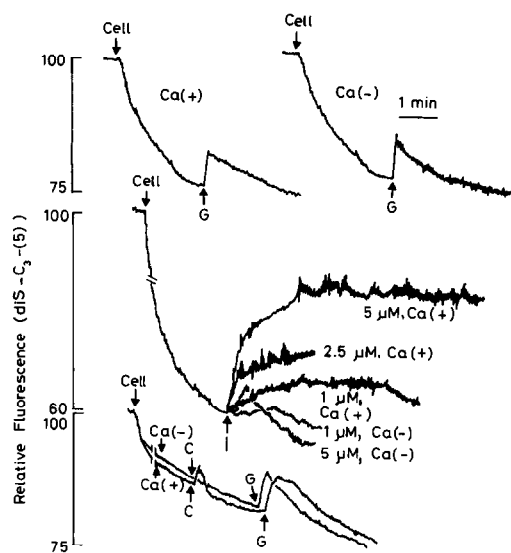


Fig.1. Membrane polarization of the parietal cell as measured by diS-C<sub>3</sub>-(5). G, gastrin ( $10^{-8}$  M); I, ionomycin (concentrations as indicated); C, carbachol ( $10^{-4}$  M). The ligand was added 3 min after the cells. Ca(+) and Ca(-): 1 mM  $Ca^{2+}$  and 0 mM  $Ca^{2+}$  plus 1 mM EGTA in the medium, respectively (including figs 2,3). Cells added to 1  $\mu$ M diS-C<sub>3</sub>-(5) in standard medium. The trace is representative of at least five similar experiments.

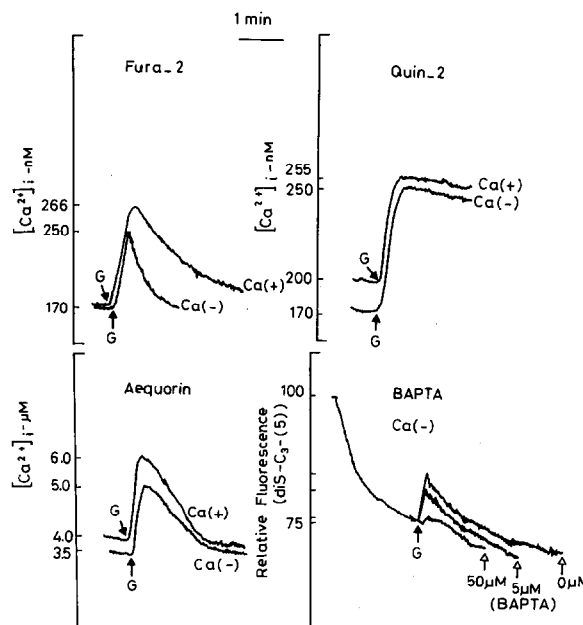


Fig.2.  $[Ca^{2+}]_i$  and membrane polarization in response to gastrin. The cells were with BAPTA acetoxy methyl ester at varying concentrations for 10 min at 37°C before stimulation. G, gastrin ( $10^{-8}$  M). The trace is a representative of at least five similar experiments.

caused a rapid but transient increase in  $[Ca^{2+}]_i$  even in the absence of medium  $Ca^{2+}$  (about 60–90 nM final rise). It seems unlikely that the luminescence in the resting state as measured using aequorin-loaded cells with high  $[Ca^{2+}]_i$  is due to their increased permeability, since they contain micromolar  $[Ca^{2+}]_i$  even in the absence of medium  $Ca^{2+}$ , thus suggesting a different  $Ca^{2+}$  gradient in the cytosol and heterogeneity of  $[Ca^{2+}]_i$  [14]. Gastrin led to an increase in  $[Ca^{2+}]_i$  which reached 2.2  $\mu$ M final rise in the absence of medium  $Ca^{2+}$ , a value which coincided with that obtained in the presence of medium  $Ca^{2+}$ . These results indicate that gastrin induces intracellular  $Ca^{2+}$  release from the store(s). Therefore, the experiments mentioned below were performed under the conditions of a  $Ca^{2+}$ -free medium. The incorporated  $Ca^{2+}$ -chelating agent, BAPTA acetoxymethyl ester (50  $\mu$ M), which enters the cytosol [17], strongly inhibited the gastrin-induced increase in diS-C<sub>3</sub>-(5) fluorescence. BAPTA entering the cytosol did not influence the resting diS-C<sub>3</sub>-(5) fluorescence. Therefore, intracellular  $Ca^{2+}$  released from the store(s) during gastrin stimulation may regulate membrane depolarization.

As shown in fig.3, changes in membrane potential could be recorded in fura-2-loaded cells since there was no interference between the fluorescence from fura-2 and diS-C<sub>3</sub>-(5). The gastrin-induced

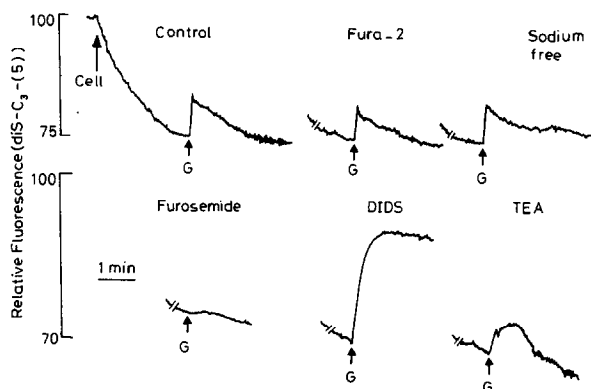


Fig.3. Effects of fura-2, medium  $Na^+$ , furosemide (1 mM), DIDS (0.2 mM) and TEA (5 mM) on gastrin-elicited membrane polarization. Pretreatment of cells with the indicated ligands was performed for 3 min at 37°C before gastrin stimulation. Medium  $Ca^{2+}$  was zero (0 mM  $Ca^{2+}$ , 1 mM EGTA). The trace is representative of at least five similar experiments.

membrane depolarization was strongly inhibited by pretreatment of cells with 1.0 mM furosemide but not with 5.0 mM tetraethylammonium (TEA) and with  $2 \times 10^{-4}$  M 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). In particular, pretreatment of cells with DIDS caused a rapid but large increase in diS-C<sub>3</sub>-(5) fluorescence after gastrin stimulation without inducing the following re-polarization. The omission of medium  $Na^+$  under conditions of equal ionic strength using choline failed to inhibit the gastrin-induced depolarization.

#### 4. DISCUSSION

This study has elucidated the role of  $Ca^{2+}$  metabolism during gastrin stimulation under the cascade of steps of the acid secretory process. As shown in previous studies and the present work [1,2], gastrin led to an increase in  $[Ca^{2+}]_i$  due to  $Ca^{2+}$  released from the store(s), presumably the smooth-surfaced membrane in the apical portion of the gastric parietal cell. Furthermore, gastrin led to membrane depolarization even in the absence of medium  $Ca^{2+}$  as measured with diS-C<sub>3</sub>-(5). The results suggest that there is a close relationship between intracellular  $Ca^{2+}$  metabolism and membrane depolarization during gastrin stimulation. The incorporated  $Ca^{2+}$  chelator BAPTA inhibited gastrin-induced membrane depolarization in the absence of medium  $Ca^{2+}$ . This in turn suggests that the  $Ca^{2+}$  released from store(s) regulates membrane depolarization. BAPTA also inhibited the increase in the fura-2 signal caused by gastrin (not shown). The evidence that  $Ca^{2+}$  regulates membrane depolarization was further supported by the fact that in the presence of medium  $Ca^{2+}$ , the  $Ca^{2+}$  ionophore ionomycin caused a large increase in membrane depolarization without promoting the subsequent re-polarization since no  $Ca^{2+}$  gradient was formed [2]. It seems unlikely that the gastrin-elicited depolarization is due to  $Na^+$  entry into the cell from the extracellular space, since the omission of medium  $Na^+$  by substitution with choline had no influence. In contrast, the reduction or omission of medium  $Na^+$  enhanced the gastrin-induced increase in  $[Ca^{2+}]_i$ , possibly owing to the cessation of  $Ca^{2+}$  efflux across the plasma membrane by  $Na^+$ - $Ca^{2+}$  antiport [2]. On the other hand, in the

apical surface of the parietal cell a  $\text{Cl}^-$  conductance is present in the stimulated vesicles but not in the resting state which is distinct from a  $\text{K}^+$  conductance [7,8] and could be selectively blocked by furosemide which is regarded as an anion carrier blocker in other systems [18].  $\text{K}^+$  and  $\text{Cl}^-$  pathways are separable and the  $\text{Cl}^-$  component in the stimulated state is more severe [7,8].  $\text{K}^+$  diffusion potentials are also present in both resting and stimulated vesicles. The gastrin-induced depolarization was clearly sensitive to furosemide but not TEA which is regarded as a  $\text{K}^+$  channel blocker [19], thus suggesting that the  $\text{Ca}^{2+}$  released from the apical store(s) during gastrin stimulation regulates  $\text{Cl}^-$  efflux across the luminal surface, possibly via (a)  $\text{Cl}^-$  channel(s), which occur(s) separately from  $\text{K}^+$  efflux, leading to membrane depolarization. The anion exchanger, i.e.  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchanger, of the parietal cell is inhibited by DIDS [20]. A secondary or indirect effect of stimulation seems to be increased  $\text{Cl}^-$  uptake across the basolateral membrane via  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange [7]. If DIDS acts inhibitorily upon a  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchanger, the failure of  $\text{Cl}^-$  replenishment could occur resulting in a significant loss of cellular  $\text{Cl}^-$  by the gastrin-induced  $\text{Cl}^-$  efflux across the luminal surface and in enhancement of gastrin-elicited membrane depolarization.

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